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Increasing the Usefulness of Acute Toxicity Tests

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C. E. Stephan[1]

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ABSTRACT: The conceptually and practically simple acute toxicity test provides information that is useful in the protection of aquatic life from pollution. Standardization increases the usefulness of routine acute tests by increasing their quality and comparability and allowing the study of various aspects of acute toxicity. For example, available data show that for certain materials some species of aquatic animals are at least 5000 times more acutely sensitive than other species, whereas for other materials the range of acute sensitivity is less than a factor of 50. Various theoretical interpretations of the relationship of toxicity to time within a test have been proposed to improve the usefulness of the acute test, but such theories have not been supported by adequate pertinent data. The usefulness of routine acute toxicity tests can be increased, however, by basing the results not on one effect or another but on a combination of all severe adverse effects, such as death, immobilization, and loss of equilibrium.

KEY WORDS: aquatic toxicology, water pollution, toxicity test, water analysis, aquatic toxicity, acute toxicity, hazard assessment

Some people feel that the only good aquatic toxicity test is a chronic test, although they usually accept acute tests that are good predictors of chronic toxicity. Others feel that the acute test is too long and costly and needs to be replaced by tests that last no longer than a few hours. Despite these attitudes, the ordinary acute toxicity test is alive and well in aquatic laboratories around the world because it is much simpler and cheaper to conduct than a chronic test, it can be adapted easily to a wide variety of important and unimportant aquatic animals, and the results are usually based on important adverse effects and have both relative and absolute significance.

[1] Environmental scientist, U.S. Environmental Protection Agency, Environmental Research Laboratory–Duluth, Duluth, Minn. 55804.

Usefulness of Acute Tests

The usefulness of the simple acute toxicity test is obvious to anyone who has tried to assess hazard to aquatic organisms or who has tried to derive water quality criteria for the protection of aquatic life. In addition, results of acute toxicity tests are directly applicable to deriving mixing zone standards, to predicting the effects of spills, and to determining maximum allowable concentrations of pollutants in bodies of water. If useful application factors or acute–chronic ratios are available, meaningful predictions of chronic toxicity can be made on the basis of the results of acute tests.

The availability of 64 water quality criteria documents was announced in the *Federal Register* on 28 Nov. 1980 (pp. 79318–79379), and the aquatic life portions of these documents rely heavily on the results of acute toxicity tests to derive criteria for aquatic life. Not only were the results of acute tests individually useful, but the set of results available for each pollutant was valuable in establishing the range of the acute sensitivities exhibited by various aquatic animal species to the individual pollutants, as shown in Table 3 of the aquatic life sections. A simple way to express this range is the high–low factor, which is obtained by dividing the acute value for the most resistant freshwater animal species for which pertinent data are available by the acute value for the most sensitive freshwater animal species. Comparable factors can be calculated for saltwater animals. For 21 pollutants acute values are available for species in at least eight freshwater families; for 17 pollutants values are available for species in at least eight saltwater families (Table 1). Of these 38 factors, 4 were above 5 000 and 5 were below 50. The largest factor was 21 600, and the smallest was 21. Not only were some of the factors very large, but the range of the factors was also large. This means that a tenfold difference in concentration of one pollutant might be crucial to almost 50 percent of the species, but for another pollutant it might be crucial to less than 1 percent.

Most of these data sets are for metals and pesticides. Although the number of species in a set ranges from 8 to 45, this does not seem to affect the high–low factor. Both freshwater and saltwater high–low factors are available for 17 pollutants, and one factor is sometimes more than 50 times the other. The saltwater factor is much higher than the freshwater factor about as often as the reverse is true. Some preliminary analyses indicate that significant contributions to the individual factors come from differences in sensitivity between species in a family, between families in a class, and between classes. Undoubtedly sets of data like these can provide information on other aspects of acute toxicity, but my purpose is merely to show one important practical use of the results of acute toxicity tests, beyond the common direct applications.

TABLE 1—*High–low factors for various pollutants.*

Pollutant	Water	Number of Species	Acute Values, μg/litre		High–Low Factor
			Highest	Lowest	
Aldrin	fresh	21	19 000	4	4 750
	salt	16	100	1.5	67
Arsenic	fresh	12	41 760	812	51
Cadmium	fresh	29	138	0.02	6 900
	salt	31	50 600	41.3	1 225
Chlordane	fresh	14	190	3	63
	salt	8	120	0.4	300
Chromium III	fresh	18	1 075	33.4	32
Chromium VI	fresh	14	195 000	67	2 910
	salt	19	105 000	2 000	52
Copper	fresh	45	260	0.23	1 130
	salt	22	600	28	21
Cyanide	fresh	15	2 326	57	41
DDT	fresh	42	1 230	0.36	3 420
	salt	17	89	0.14	636
Dieldrin	fresh	19	740	2.5	296
	salt	21	50	0.7	71
Endosulfan	fresh	10	261	0.34	768
	salt	12	730	0.04	18 250
Endrin	fresh	28	352	0.15	2 350
	salt	21	14.2	0.037	384
Heptachlor	fresh	18	320	0.9	356
	salt	19	194	0.057	3 400
Lead	fresh	9	815	1.16	703
Lindane	fresh	22	676	2	338
	salt	19	3 680	0.17	21 600
Mercury	fresh	11	2 000	5[a]	400
	salt	26	1 680	3.5	480
Nickel	fresh	22	2 230	54	41
	salt	17	350 000	152	2 300
Selenium	fresh	13	42 400	340	125
	salt	13	17 348	599	29
Silver	fresh	10	5.77	0.00192	3 000
	salt	10	1 400	4.7	298
Toxaphene	fresh	29	180	1.3	138
	salt	14	1 120	0.11	10 200
Zinc	fresh	29	2 260	8.89	254
	salt	24	70 600	166	425

[a]The acute value for *Faxonella clypeata* in Criterion Document should have been 20 μg/litre, not 0.02 μg/litre (from a letter to J. H. McCormick, U.S. Environmental Protection Agency, by M. Heit, U.S. Energy Research and Development Administration, 27 Jan. 1981).

Increasing Usefulness Through Standardization

A significant increase in the usefulness of acute toxicity tests is possible through consensus standardization of methodology and terminology. Through consideration of ideas and data from a large number of people, consensus standardization should result in adoption of the best available procedures, reduce the number of poor quality tests, and increase the number of valid comparisons. In addition, standardization often encourages research because points which are important and controversial enough to deserve additional study are identified in the process of writing methods. After a method is written, it provides a basis against which suggested improvements can be compared.

Standardization could decrease the usefulness of the acute test if it were applied so rigidly that people were forced to conduct tests using inappropriate methods. If the test material or species requires a special procedure, that procedure should be allowed. But this should not be interpreted as a license to conduct every test in a new and different way, and the mere use of a novel procedure should not be considered research. Good research must allow valid comparisons of results, and the continuous use of new and different methods does not allow this.

Standardization of the acute toxicity test may seem unnecessary because current methods are not drastically different from those described in 1945 in the classic publication by Hart, Doudoroff, and Greenbank [*1*].[2] However, flow-through acute tests have become common only in the last 20 years, data analysis has become sophisticated, and analytical chemistry is now routinely used. Although more and more people are conducting acute tests, standardization efforts have resulted in more agreement as to how such tests should be conducted, both in principle and in practice.

Adoption of appropriate terminology also helps increase the usefulness of acute tests by making it easier to communicate concerning methodology and results. The term "median tolerance limit" (TL_m, TL_{50}) and the occasionally misused term "median lethal dose" (LD_{50}) have been almost completely replaced by "median lethal concentration" (LC_{50}), thanks largely to Sprague [*2*].[3]

[2] The italic numbers in brackets refer to the list of references appended to this paper.

[3] Sprague [*2*] also argued that the "50" should not be a subscript, but should be on the line as originally proposed by Trevan [*3*]. Making the 50 a subscript serves no useful purpose but does unnecessarily complicate the lives of authors and typists. Hayes [*4*] pointed out that Trevan [*3*] also placed a space before the 50, which improves clarity in some situations. Although use of the 50 on the line is increasing [for example, see Refs *4* through *11*, *Toxicology and Applied Pharmacology* (Information for Authors), *Federal Register*, 28 Nov. 1980, pp. 79341–79347, the ASTM Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs (E 724–80), and the ASTM Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (E 729–80)], some opposition still exists [*12*].

It would also be appropriate to replace the term "bioassay" with "toxicity test," because these tests almost always measure toxicity, a property, and do not determine what portion of a sample consists of a specific element, ion, or compound [*13,14*]. Further, in the last few years the word bioassay has been more and more associated with studies of carcinogenicity. Even though this is another deviation from the classic meaning of the word, the increasing association of the term bioassay with carcinogenicity is one more reason for aquatic toxicologists to avoid use of this word for toxicity tests.

Similarly, in toxicology it is more appropriate to speak of the *effect* of the test material on the organism than the *response* of the organism to the material. For example, we should use the term "concentration–effect curve" rather than "concentration–response curve," and we should say that different organisms are affected in different ways, rather than that different organisms respond in different ways. When a test organism is exposed to a lethal concentration of test material, the organism does not respond by dying; it fights to live. Most organisms respond to stress with one or more defense mechanisms, but death is not one of them. Death is not a *response by* an organism; rather, it is an *effect on* an organism. The organism does not respond by dying, reducing growth, or becoming immobilized. The organism is killed, stunted, or paralyzed by the test material. This is why we speak of the "median effective concentration" (EC_{50}) and "adverse effects" rather than the "median response concentration" (RC_{50}) and "adverse responses." We have switched from "dose–response curve" to "concentration–response curve," and now we should go the next step to "concentration–effect curve."

Increasing Usefulness Through Toxicity Curves

It has been suggested that the usefulness of the acute test can be increased by taking advantage of various proposed relationships between the EC_{50} and the time during a test. (The term EC_{50} is used herein to include all adverse effects, including death, and so all of the following also pertains to LC_{50} values.) Several forms for the toxicity curve have been proposed [*7,15–21*] using various combinations of logarithms, reciprocals, powers, and constants. The usual suggestion is that a specified curve be fitted through the EC_{50} values obtained at various times during a test so that constants or intercepts can be calculated; most such suggestions are accompanied by at least one set of data which the suggested curve fits well. Usually the major purpose of the calculation is to extrapolate to the concentration that would affect half of the test organisms during an extended exposure. Several names have been proposed for this concentration (when the effect is death), such as incipient lethal level, ultimate TL_m, lethal threshold concentration, asymptotic LC_{50}, and infinity concentration [*2,22*]. The term "asymptotic EC_{50}"

avoids some problems [*8*] and would seem to be a very appropriate term when a technique is used to extrapolate to an assumed asymptote.

In 1968 I proposed that the "time-independent TL_m" be defined as the TL_m that does not change by more than 10 percent in 24 h [*23*]. This kind of definition avoids the problem of trying to describe the shape of the whole toxicity curve by dealing only with the end of the curve. Although both the term "time independent" [*9,24*] and the definition [*25*] have been used, the term was probably inappropriately used and the definition should be avoided altogether. When an EC_{50} is obtained by extrapolation [*9,24*] to infinite time, it should be called an asymptotic EC_{50}. The term "time-independent EC_{50}" should be applied only to an EC_{50} that has been experimentally shown to be independent of time. An EC_{50} should be called time independent only when data show that the EC_{50} did not change by more than a small percentage over a reasonably long period of time during a test. A decrease of 10 percent in 24 h cannot be considered time independent. One percent in 24 h might not even be small enough because this adds up to 30 percent per month.

Unfortunately, the experimental designs generally used for acute toxicity tests today [see the ASTM Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians (E 729–80)] are not stringent enough to provide definitive information about the shape of the toxicity curve or the existence of a time-independent EC_{50}. The test concentrations are spaced so far apart and so few organisms are used at each concentration that some tests appear to produce a time-independent EC_{50} merely because small changes in the EC_{50} cannot be detected in short periods of time. The problem is to decide what is the maximum acceptable rate of change and then devise an appropriate experimental design. Acute tests would have to be designed so that aquatic toxicologists could be confident that the actual, not just the apparent, rate of change did not exceed the maximum allowed. Unfortunately, if the experimental design were improved and the definition of "time independent" were stringent, fewer tests would produce a time-independent EC_{50}, and of course this whole idea is useless for all tests in which the EC_{50} does not become time independent.

Various kinds of theoretical definitions have been proposed for the asymptotic or time-independent EC_{50}. One suggestion is that it is the highest concentration at which half the test organisms would survive indefinitely. This ignores the fact that no test organism will survive indefinitely at any concentration. More important, it ignores the possibility of chronic effects on survival. Another suggestion is that an asymptotic or time-independent EC_{50} is the concentration at which acute toxicity ends. This suggestion is essentially an operational definition of acute toxicity and, therefore, cannot be disproved. However, it implies that, if no asymptotic or time-independent EC_{50} is observed, acute toxicity continues for a long time; but by definition toxicity which does not occur in a short time cannot be considered acute.

The major argument advanced for the determination of an asymptotic or time-independent EC_{50} is that it is a better measure of acute toxicity than an EC_{50} measured at some prespecified time, such as 48 or 96 h. This theory sounds good, but there is no general application for which an asymptotic or time-independent EC_{50} has been shown to be more useful than a 48 or 96-h EC_{50}, depending on the test species. The extra cost of conducting more precise or longer tests is not justified by any practical benefit. Why be very precise about one species when the next species tested may be 50 or 5000 times more or less sensitive? It is not a good use of resources to conduct a test for up to 30 days [*9*] just to measure survival, when a 30-day test on some species of fish [*10*], daphnids, and mysid shrimp can give a good estimate of effects on survival, growth, and reproduction.

Another major problem with the theory of the importance of an asymptotic or time-independent EC_{50} is that the theory does not apply to all acute tests. What is to be reported for tests in which the EC_{50} continually decreases as the length of the test increases? If the criterion for goodness of fit is reasonably stringent for calculation of an asymptotic EC_{50} or if the definition of a time-independent EC_{50} is reasonably stringent, it is likely that quite a few acute tests will produce neither an asymptotic nor a time-independent EC_{50}.

Cardwell et al [*11*] obtained linear, hyperbolic, and sigmoid toxicity curves in tests on a variety of test materials with up to six species of fish. For three of these materials, time-independent EC_{50}s were observed with some species but not with others. The results may indicate that no one curve has very wide applicability. Of course, because of the experimental design, it is difficult to determine if one curve fits the data significantly better than another. Even more variety may have been observed if tests had been conducted with invertebrate species.

Because most of the theories concerning the shapes of acute toxicity curves are not well supported by data, I wish to propose another theory which fits the existing data as well as any existing theory. This proposal is that acute toxicity curves follow a generalized pattern (Fig. 1, solid line), but not a specific form. From one test to another all that changes are the slopes of the segments and the relative positions of Points A to E, that is, the durations of the various phases. This flexibility makes it possible to fit this kind of curve to almost any set of data. With fish, 96 h often occurs between Points C and D. The results of Cardwell et al [*11*] show that it is not unusual for an apparently time-independent EC_{50} to last for a short time and be followed by additional mortality after Point D. In such cases a time-independent EC_{50} may be just an artifact of the experimental design or the length of the test. This type of segmented curve can fit several different types of observed toxicity curves, such as curvilinear (A–B), linear (B–C), asymptotic (A–D), and nonasymptotic (A–E), depending on which segments and durations are used.

If a time-independent EC_{50} is observed, that is, if Points C and D are very far apart, the toxicity curve can just as easily be approximated by a rectangu-

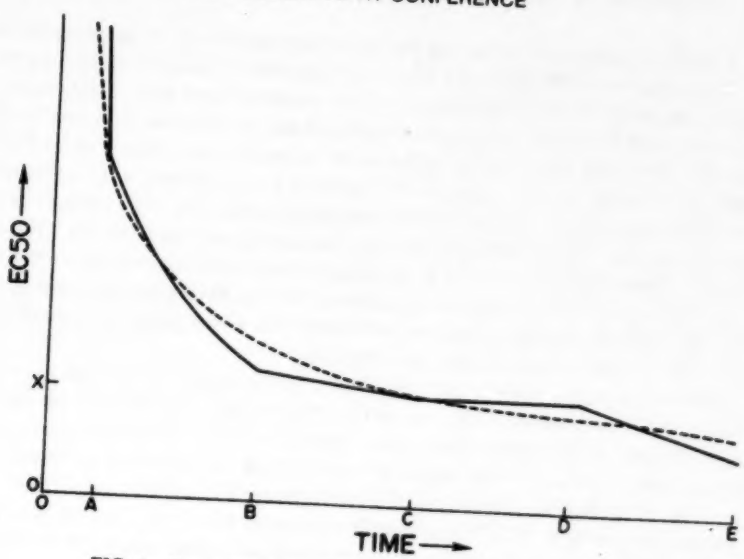

FIG. 1—*Generalized plots of EC_{50} versus time in acute toxicity tests.*

lar hyperbola with a latent time of *A* and a time-independent EC_{50} of *X*. The same is true for almost any test which is stopped before Point D. Unfortunately, it is very difficult to distinguish toxicity curves represented by the dashed and solid lines in Fig. 1 unless the test concentrations are close together and numerous test animals are exposed to each treatment. A test in which no additional organisms are affected between 24 h and 14 days is easily distinguished from a test in which several additional organisms are affected every day of the test. However, numerous tests fall into the "gray zone," and the apparent existence of a time-independent EC_{50} is almost totally dependent on the experimental design and the definition of time independent.

This theory, because it is so flexible, has no practical use. The only reason for presenting it is to emphasize that conclusions and extrapolations based on the shape of the toxicity curve are supported by theories, not definitive data. If the segmented curve theory is true, other proposed curves cannot have any utility. Whether a test which actually has a toxicity curve like either the solid or dashed line is reported as having a time-independent EC_{50} will depend on the definition employed, the judgment of the investigator, and the concentrations actually used in the toxicity test. Both the experimental design and the kind of test used may affect the apparent existence of a time-independent EC_{50}. For example, with volatile and degradable materials static tests may be more likely to produce a time-independent EC_{50} than flow-through tests. This is still very nebulous because few good comparisons of

the results of comparable static and flow-through tests are available. Intuitively, it would seem that volatile and hydrolyzable materials should appear to be less toxic in static tests than in flow-through tests. However, other factors such as the rate of effect may be as important, and therefore some comparisons should be performed.

From a practical standpoint a time-independent EC_{50} is no more useful than an EC_{50} measured at a time appropriate for the test species. Today there seems to be general agreement as to how long acute tests with most species ought to last. A few years ago some acute tests with fish lasted only 24 h, and many tests lasted only 48 h. Most people now feel that, except in special cases, acute toxicity tests with fish should last at least 96 h to obtain a good measure of acute toxicity. Although it can be argued that 96 h was chosen as a matter of convenience, it can also be argued that 24 or 48 h would be more convenient. The major reason for acute tests lasting 96 h with fish and many other aquatic animals is that the toxicity curve often appears to be relatively flat at about 96 h, even if additional organisms are affected after 96 h. If a test is terminated when the EC_{50} is changing too rapidly, the result will be too dependent on the duration of the test. In addition, in static tests it is often difficult to maintain acceptable water quality for more than 96 h without substantially increasing the size of the system. For some life stages of some species, such as oyster larvae, midge larvae, and first instar daphnids, it does not seem necessary or appropriate to continue the test for more than 48 h. Selecting life stages and durations which are appropriate for specific species is no more arbitrary than selecting a curve to calculate an asymptotic EC_{50}, selecting a definition of time independent, or deciding how to report results of tests which do not produce an asymptotic or time-independent EC_{50}.

Other Proposed Uses for Toxicity Curves

Three other suggestions have been made for drawing conclusions from the shape of the toxicity curve. One is that an irregularity in a curve probably indicates that a change in the mode of action has occurred. Although this theory does not seem too unreasonable, to use it, one must define "irregularity" and have an experimental design that will provide good evidence as to whether an irregularity occurs. For example, the solid line in Fig. 1 contains irregularities, whereas the dashed line does not, but only an elaborate and expensive acute test could differentiate between the two. On the other hand, can a smooth curve be caused by two modes of action acting simultaneously? More important, does it really make any difference how many modes of action cause acute toxicity in a test?

Another suggestion is that test materials which produce nonasymptotic curves are cumulative poisons. Again a major problem is whether the experimental design of the acute test is sensitive enough to provide decisive information concerning the shape of the curve. And how is a test material to be

classified if a nonasymptotic curve is obtained with some species but not with others? Also, if a nonasymptotic curve is proposed as a definition of "cumulative," it should be pointed out that aquatic toxicologists use the word cumulative to describe two situations. A material is often called cumulative if, for example, the 4-day EC_{50} is substantially lower than the 1-day EC_{50}. A material is also called cumulative if exposed for 12 h every day for 8 days produce the same EC_{50} as continuous exposure for 4 days. Would materials classified as noncumulative by one definition be similarly classified according to the other definition?

A third suggestion is that a material which produces a nonasymptotic curve probably causes chronic toxicity at concentrations far below those that cause acute toxicity. Conversely, a material that produces an asymptotic curve probably does not exhibit chronic toxicity at concentrations much below concentrations that cause acute toxicity. This is potentially the most practically useful of these theoretical proposals. Unfortunately, this theory is also untested and assumes that the experimental design is good enough to define accurately the shape of the toxicity curve. Fortunately, in this case the importance of experimental design can be lessened by stating the theory, not in terms of the shape of the curve, but in terms of the "slope" of the curve, with the slope being defined as some function of the 24-h and 96-h EC_{50}s for 4-day tests, and some function of the 12-h and 48-h EC_{50}s for 2-day tests. Stated in this way, this theory is straightforward enough and so potentially useful that it ought to be tested.

Until someone demonstrates that at least one of them has some practical use, little reason exists to continue discussion of these terms, definitions, and theories. Fortunately ordinary acute tests are exceedingly useful even if they are not glamorous or complex or theoretically satisfying. And the shape of the toxicity curve can be important without the use of any of these theories. For example, if in a flow-through test the 1-h EC_{50} is the same as the 96-h EC_{50}, then a 1-h exposure will cause just as much acute toxicity as a 96-h exposure, and the 96-h EC_{50} does not provide nearly as much protection as would normally be assumed. This kind of information may be very useful in hazard assessment and regulatory decisions.

Increasing Usefulness by Means of the Concept of "Functional Death"

A basic concept of aquatic toxicology is that the acute toxicity test measures the ability of a material to affect adversely an aquatic species in a short period of time. In an acute lethality test, the adverse effect is death, and the results are usually expressed in terms of the LC_{50}. In other acute tests, a nonlethal effect is used, and the results are generally expressed in terms of an EC_{50} based on a specified effect. The effect may range from incomplete shell development for oyster larvae, which is nearly always eventually fatal, to decreased feeding for fish. Thus, results of acute tests are based on a variety of

effects that are not necessarily related to one another in any meaningful way. The routine acute toxicity test would be more useful if it were designed to measure the total acute severe adverse impact of the test material on the test organisms. Impact should be defined not in terms of one effect or another, but in terms of a combination of all those effects which are severe and adverse. Rather than an acute toxicity test with fish producing an LC_{50} based on death or an EC_{50} based on immobilization, the test might produce an EC_{50} based on death *plus* immobilization *plus* loss of equilibrium. Such an end point might be called the EC_{50} based on "functional death" and be defined as the concentration which causes severe adverse effects on 50 percent of the test organisms. (Other terms that have been used for functional death include "ecological mortality" [*26*] and "effective death" [*27*].)

Use of an EC_{50} based on functional death would allow a more realistic appraisal of the total acute severe adverse impact of the test material on the species because all those individual test animals which are dead, immobilized, or unable to maintain normal movement are, in fact, functionally dead. In a field situation, the organisms could not avoid predators and could not maintain themselves in a current. Thus, an EC_{50} based on functional death should be more meaningful than an LC_{50} or some EC_{50} for protecting organisms in mixing zones. In addition, comparisons of the sensitivities of various species to a variety of test materials would be more valid if the comparisons were based on functional death rather than on LC_{50}s or on a mixture of LC_{50} and EC_{50} values. For instance, it is not really fair to say that two materials which have identical LC_{50} values are equally acutely toxic if one, but not the other, causes immobilization at concentrations much lower than the LC_{50}. Of course, these immobilized organisms might recover after the exposure is ended, but they might just as well suffer delayed mortality or other long-term effects. More important, in a field situation these organisms would be helpless while immobilized, and they would not recover if they were eaten by a predator or harmed or injured while immobilized.

Some people feel that an LC_{50} value is a better end point than an EC_{50} because death is less subjective than effects such as immobilization. On the other hand, it is not always easy to decide when an organism is dead. The use of an EC_{50} based on functional death would increase the cost of some acute tests because investigators would have to observe the test organisms more closely. This should be considered a step in the right direction, because investigators should be encouraged to observe and report signs of stress. For many years mammalian toxicologists have considered acute tests more than just tests to see whether animals live or die. Considering the amount of time required to set up and tear down an acute test, it seems appropriate to spend a reasonable amount of time observing the test organisms.

The use of an EC_{50} based on functional death would reduce the cost of some acute tests because investigators would not have to try to differentiate between those organisms that are dead and those that are merely immobil-

ized or paralyzed. Possibly without realizing it, in tests with daphnids many people now determine a 48-h EC_{50} based on functional death rather than an EC_{50} based on immobilization or an LC_{50}, because they count both dead and immobilized animals as "affected." On the other hand, in the recently adopted ASTM Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs (E 724–80), the LC_{50} is based on death, and the EC_{50} is based on larvae that lived but did not develop complete shells. Because larvae with incomplete shells are not viable, an EC_{50} based on death and incomplete shell development would be a more rational measure of the acute toxicity of a test material to embryos and larvae of bivalve molluscs. Conversely, neither decreased shell deposition by juvenile oysters nor decreased growth by fish should be considered a severe adverse impact in acute tests.

Another common suggestion for increasing the usefulness of the acute toxicity test is to express the results in terms of an LC_5 or LC_{10} value rather than an LC_{50}. This kind of suggestion is reasonable only if an experimental design is used that produces as precise an LC_5 as is usually obtained for an LC_{50}. An EC_{50} based on functional death should be more meaningful than an LC_{50} and would not require any change in the experimental design or the analysis of the data. It does not make much sense to increase the cost of an acute test significantly to determine an LC_5 with one species if the next species may be 50 or 5000 times more or less sensitive. The use of an EC_{50} based on functional death would allow more meaningful comparisons of species and test materials with little increase, and sometimes a decrease, in the cost of acute toxicity tests.

The best routine approach to measuring acute toxicity to aquatic animals is to identify individually for each species the best life stage to test and then to identify the best length of exposure and the best combination of severe adverse effects to use in determining an EC_{50} based on functional death. (Undoubtedly many investigators would want to continue reporting LC_{50} values also.) This would not mean that other life stages, durations, and effects could not be used if desired, but it would indicate that if there were no strong reasons for doing otherwise, this is how routine acute tests with that species should generally be conducted. For many species, the end point might be the 96-h EC_{50} based on death, immobilization, and loss of equilibrium. For midge larvae and first-instar daphnids, it might be the 48-h EC_{50} based on the same effects. For bivalve mollusc larvae it might be the 48-h EC_{50} based on death and incomplete shell development. This approach would allow better comparisons between species and test materials, because comparable and appropriate life stages, durations, and end points would be used in more acute tests. Some steps have been taken in this direction in ASTM Practice E 729–80, but much more needs to be done. Toxicity tests should be designed to accommodate the test species and to satisfy the need for useful information about the effects of test materials on those species.

Fortunately, with most aquatic species it should be possible to fulfill these two requirements without too much inconvenience or cost to the investigator.

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